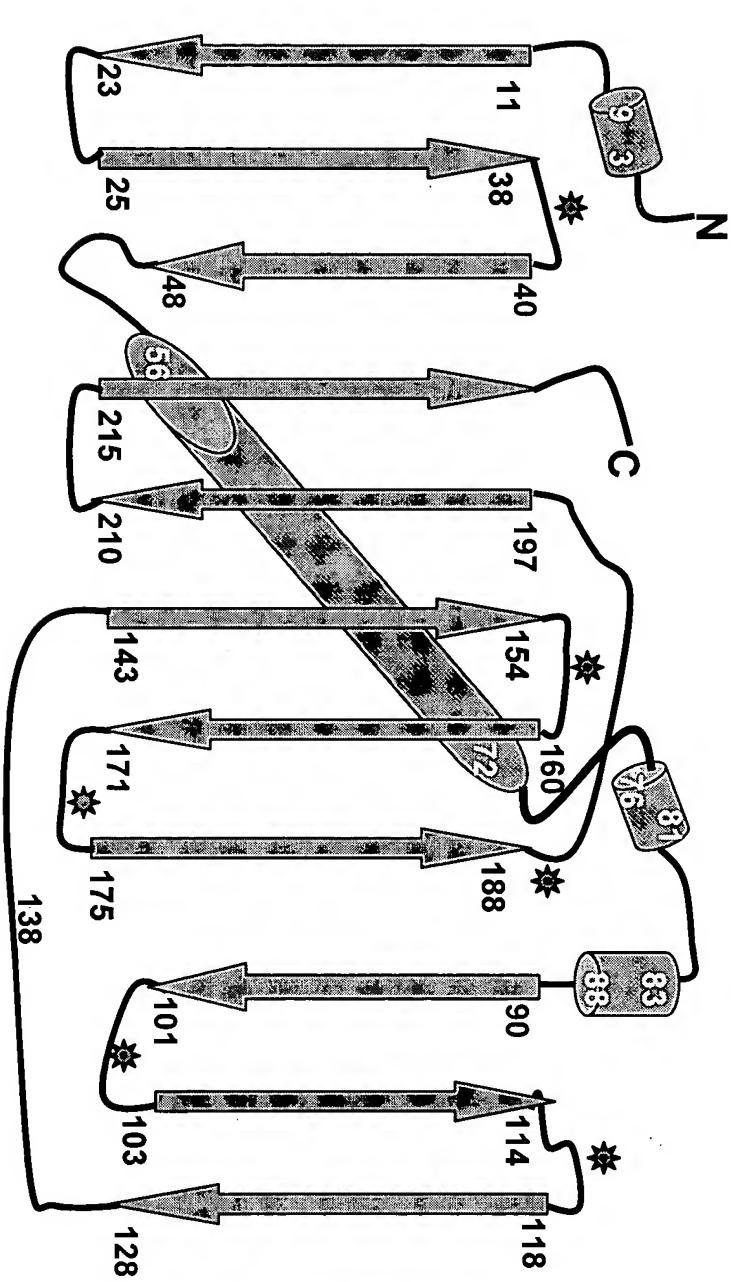


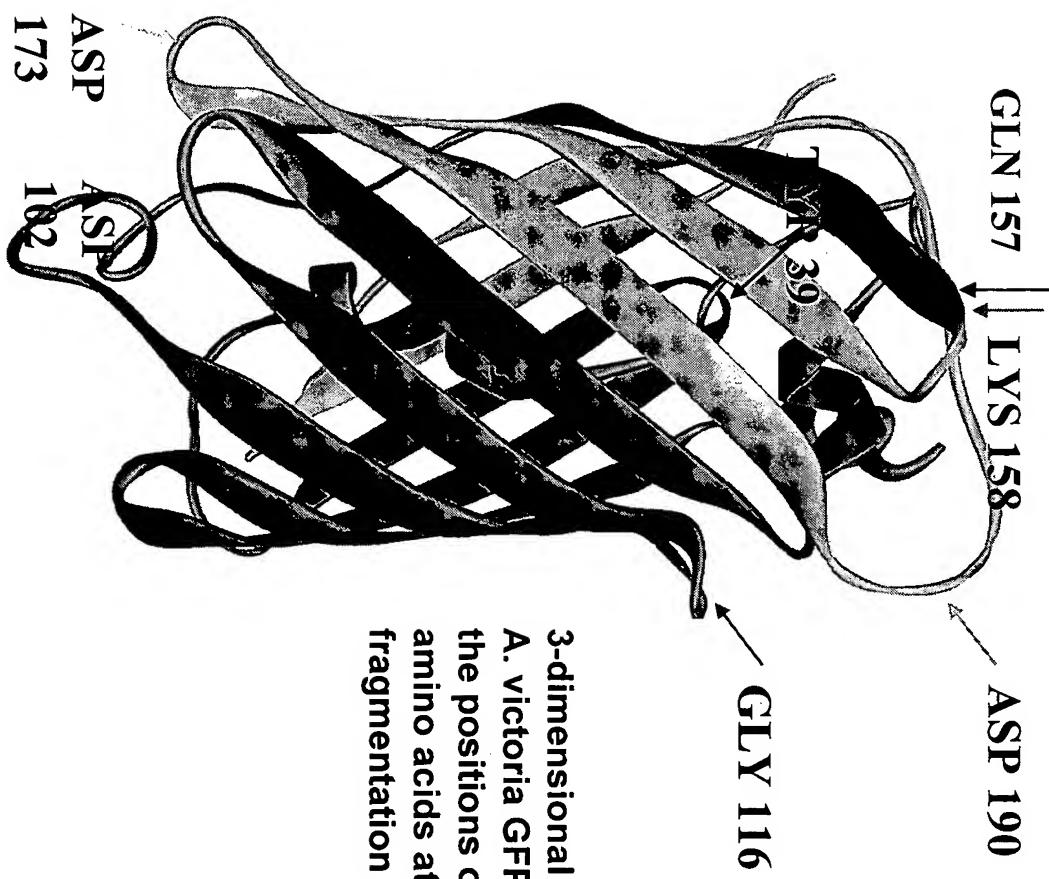
Fig. 1a



Aequorea Victoria Green Fluorescent Protein (GFP)

* Loops highlighted by asterisks designate alternative fragmentation sites that are the subject of the invention, comprising amino acid residues 38-40 (region 1); residues 101-103 (region 2); residues 114-118 (region 3); residues 154-160 (region 4); residues 171-175 (region 5); and residues 188-190 (region 6).

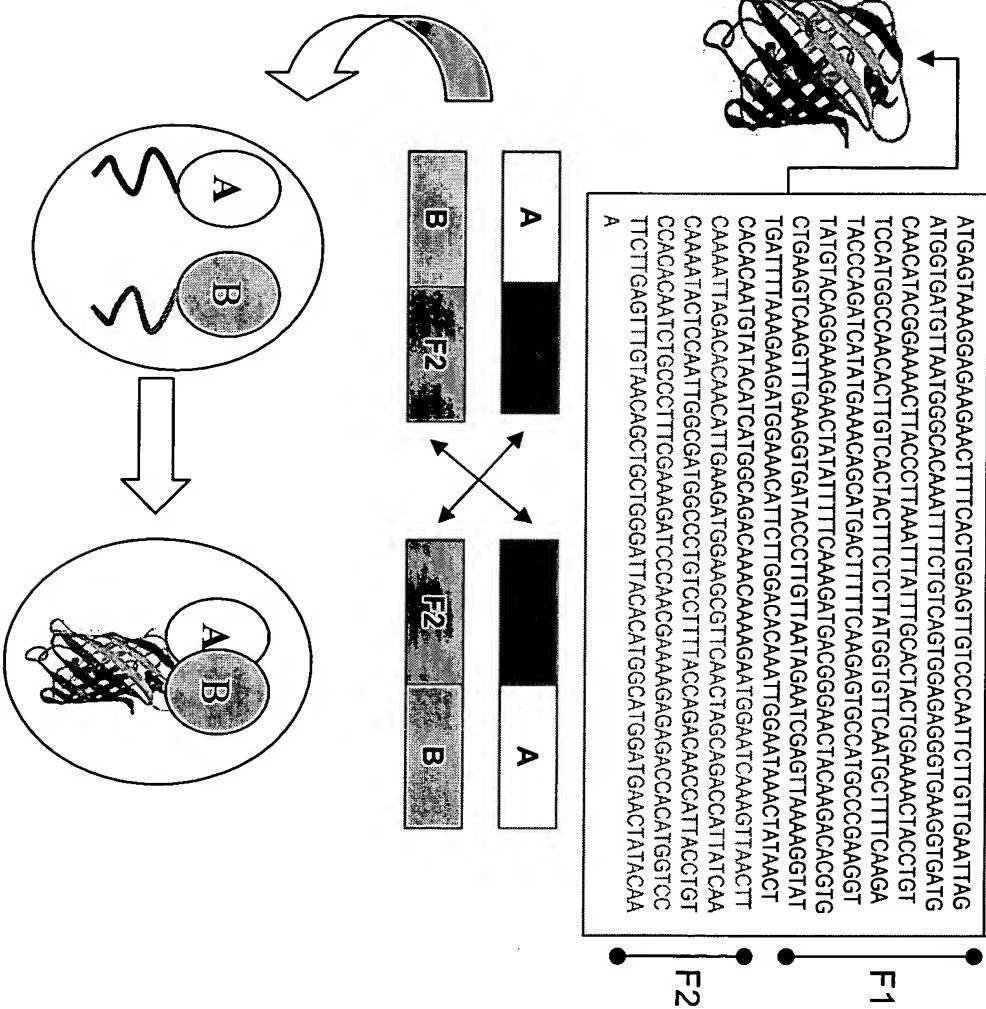
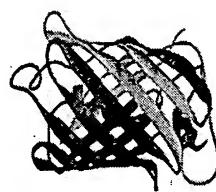
Fig. 1b



3-dimensional structure of
A. victoria GFP showing
the positions of specific
amino acids at 6 alternative
fragmentation sites

Fig. 2

1. Select a fluorescent protein and its corresponding DNA sequence
2. Create fragments and mutant fragments by:
 - A. Rational design, followed by genetic engineering or oligonucleotide synthesis; and/or
 - B. Random or directed fragmentation of full-length DNA molecule
3. Create 5' or 3' fusions of fragments (F_1, F_2) with genes encoding other proteins (A,B) in expression vectors
4. Co-transfect fragment pair fusions into cells; allow expression of fusion proteins A,B
5. Measure fluorescent signal, e.g. by fluorometry, FACS, or microscopy



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ATGAGTAAGGAGAAAGACTTTCACTGGGTTGTCCTAACATTCTGTGAATTAGATGGT
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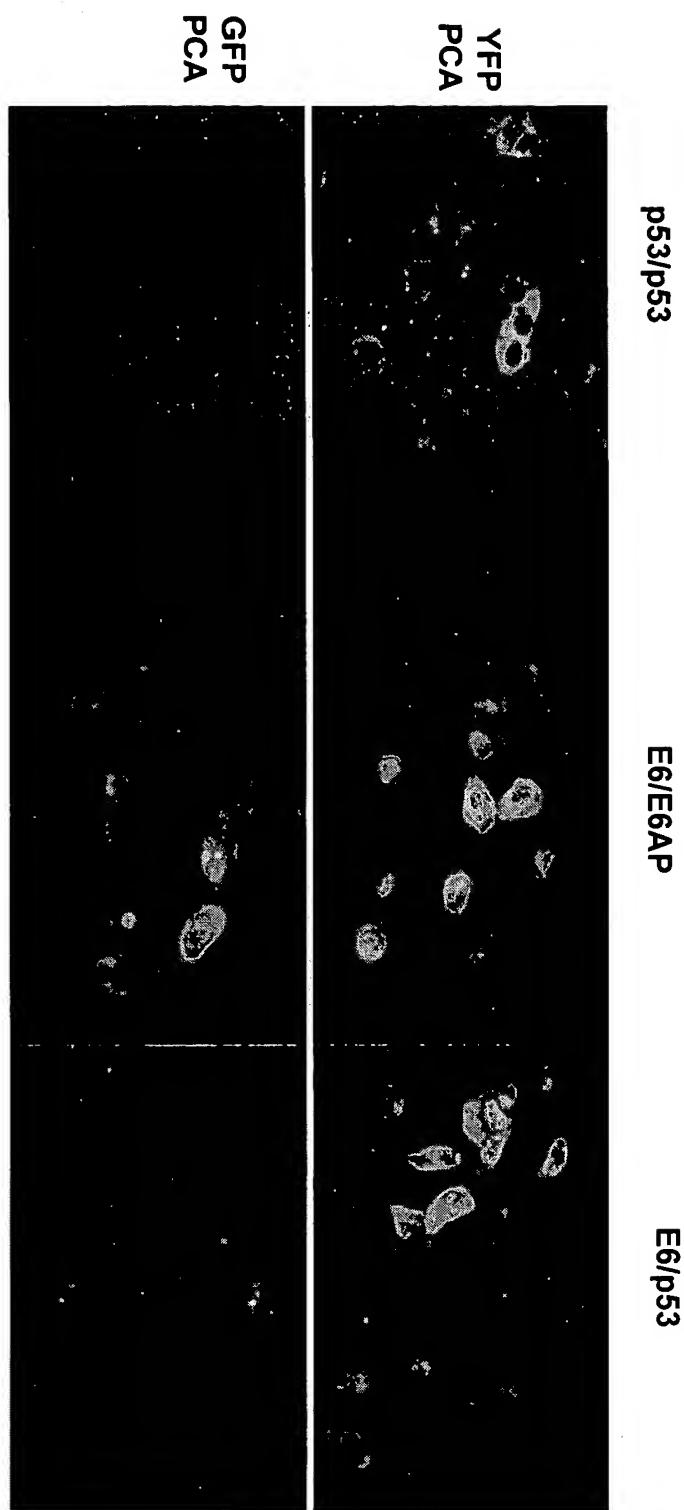
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CCACACAACTGTGCCCTTCGAAAGATCCCACAGAAAGAGAGACACATGGC
TCTTGTGAGTTAACACAGCTGGGATTACACATGGCATGGATGAACTATAA
A
  
```

F_2'

F_1

Fig. 3



24 Hour Expression in HEK293E Cells (1.2 sec exposure)

Fig. 4

Examples of PCAs based on alternative fragmentation sites of YFP

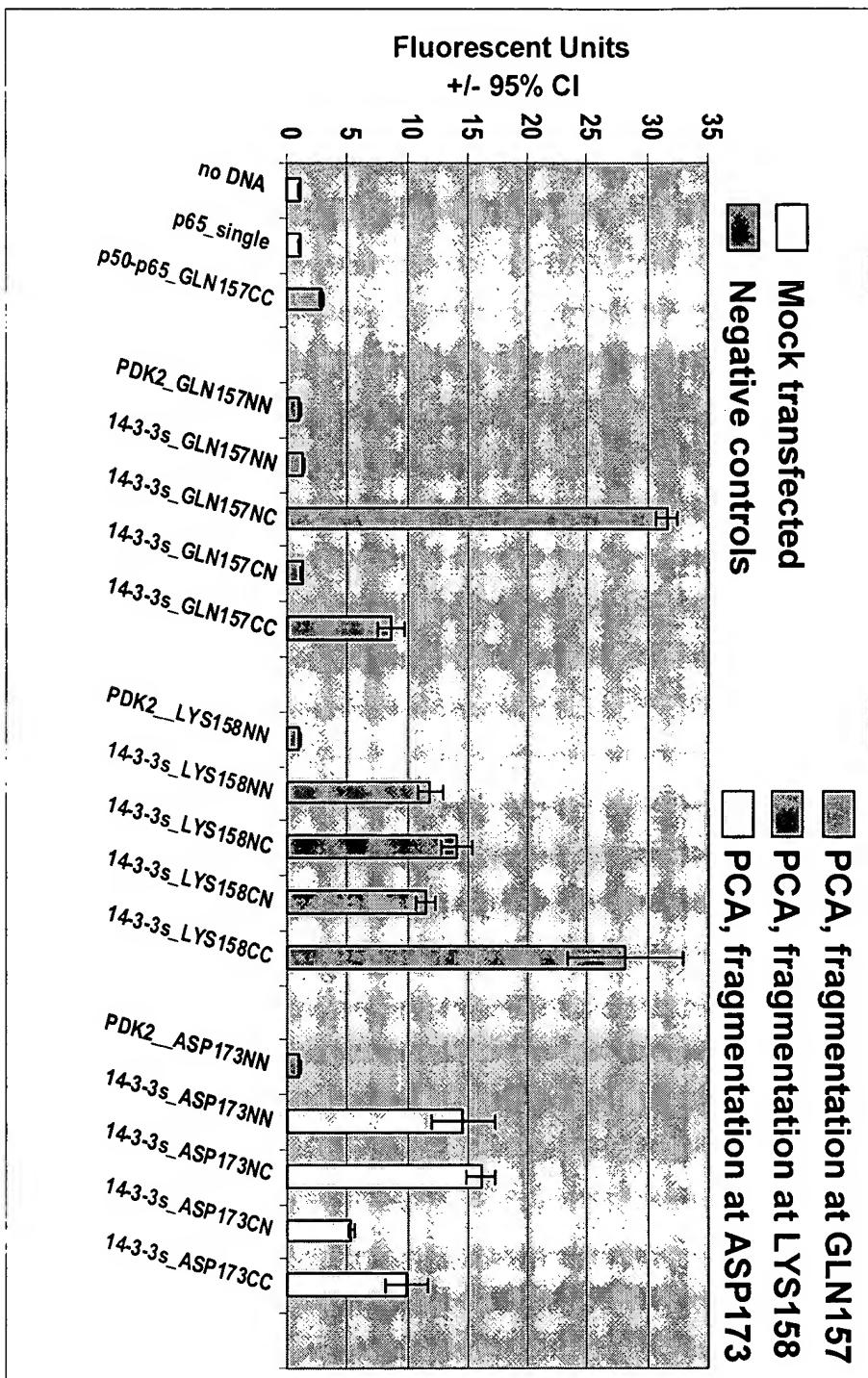
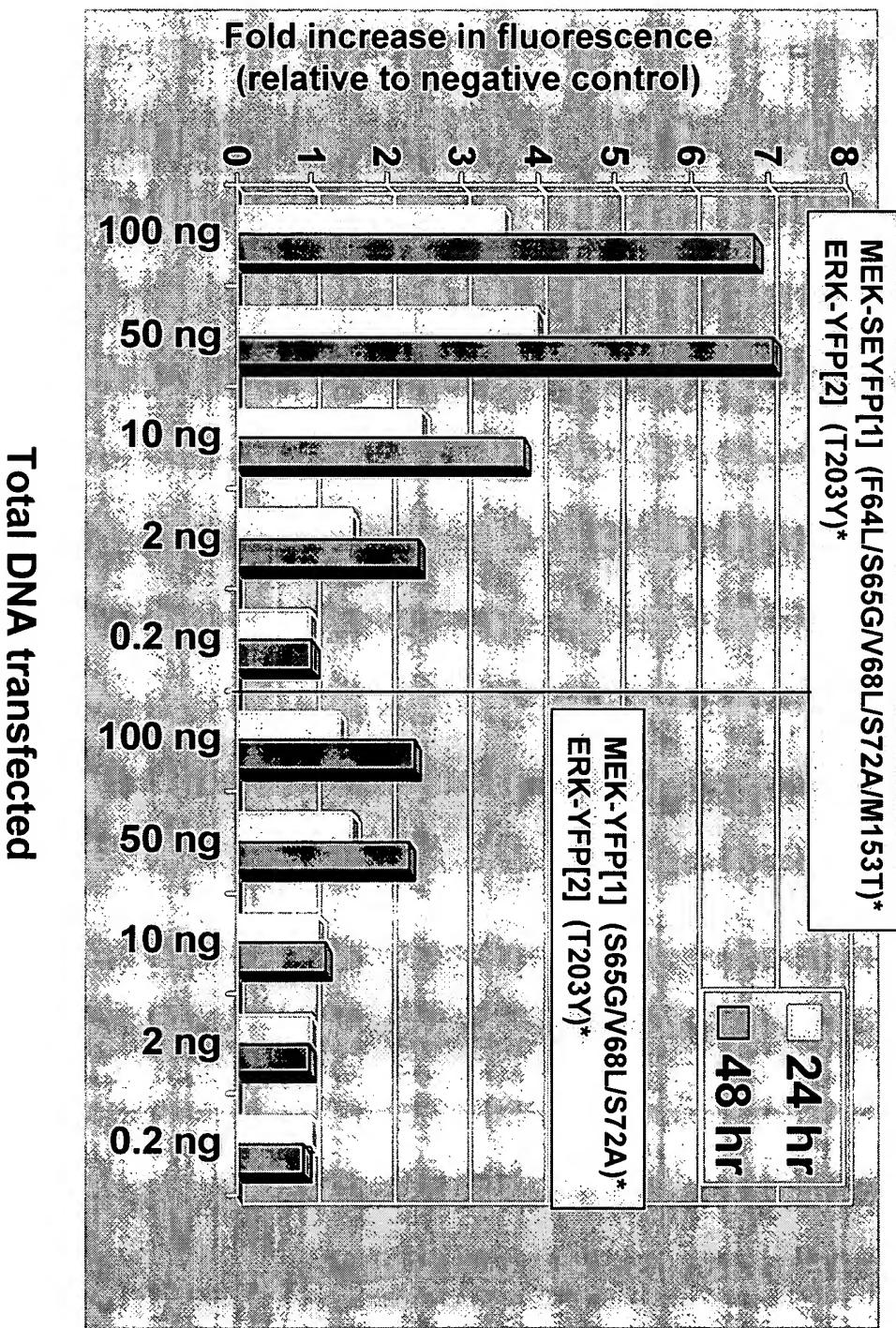


Fig. 5



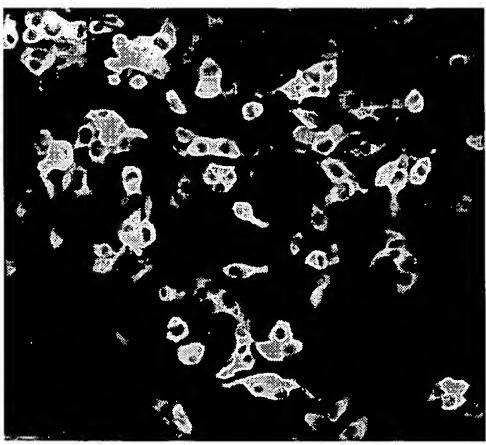
* Note: amino acid changes are designated relative to wild-type GFP

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Fig. 6

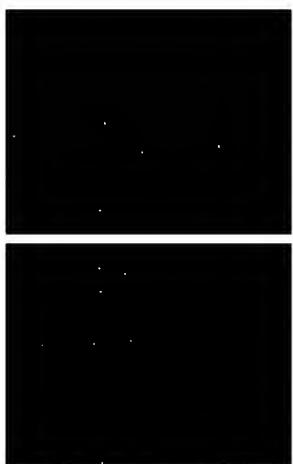
MEK-YFP[1] (F64L/S65G/V68L/S72A/M153T)*
ERK-YFP[2]-T203Y*

C. Single fragment control:
Pdk2-YFP[1] (F64L/S65G/V68L/S72A)



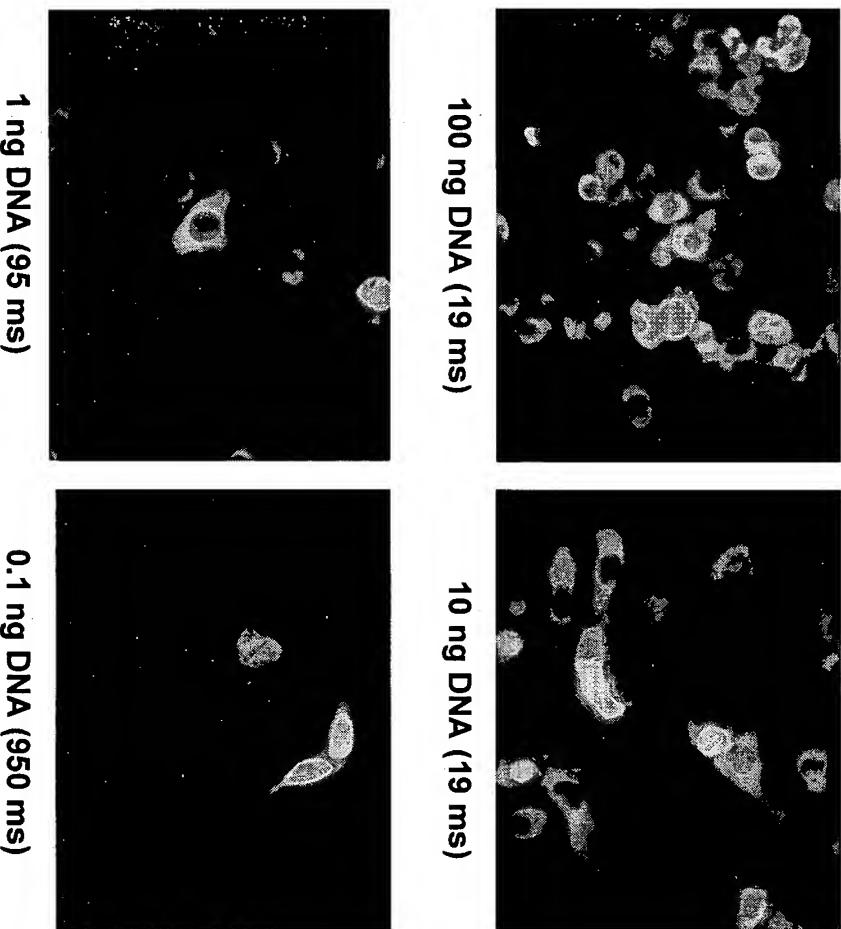
b. MEK-YFP[1] (S65G/V68L/S72A*)
ERK-YFP[2] (T203Y*)

d. Single fragment control:
Pdk2-YFP[2] (T203Y)



* Note: amino acid changes are
designated relative to wild-type GFP

Fig. 7a
An intense fluorescent PCA (IFP PCA) based on mutant fragments
of YFP



1 ng DNA (95 ms)

0.1 ng DNA (950 ms)

Results shown are for MEK-IFP[1] + IFP[2]-ERK

IFP[1]: F46L/F64L/S65G/V68L/S72A/M153T

IFP[2]: V163A/S175G/T203Y

Note: amino acid changes are designated relative to wild-type GFP

Fig. 7b.
Enhanced PCA signals based on mutant fragments:
IFP (SEYFP-F46L) versus EYFP

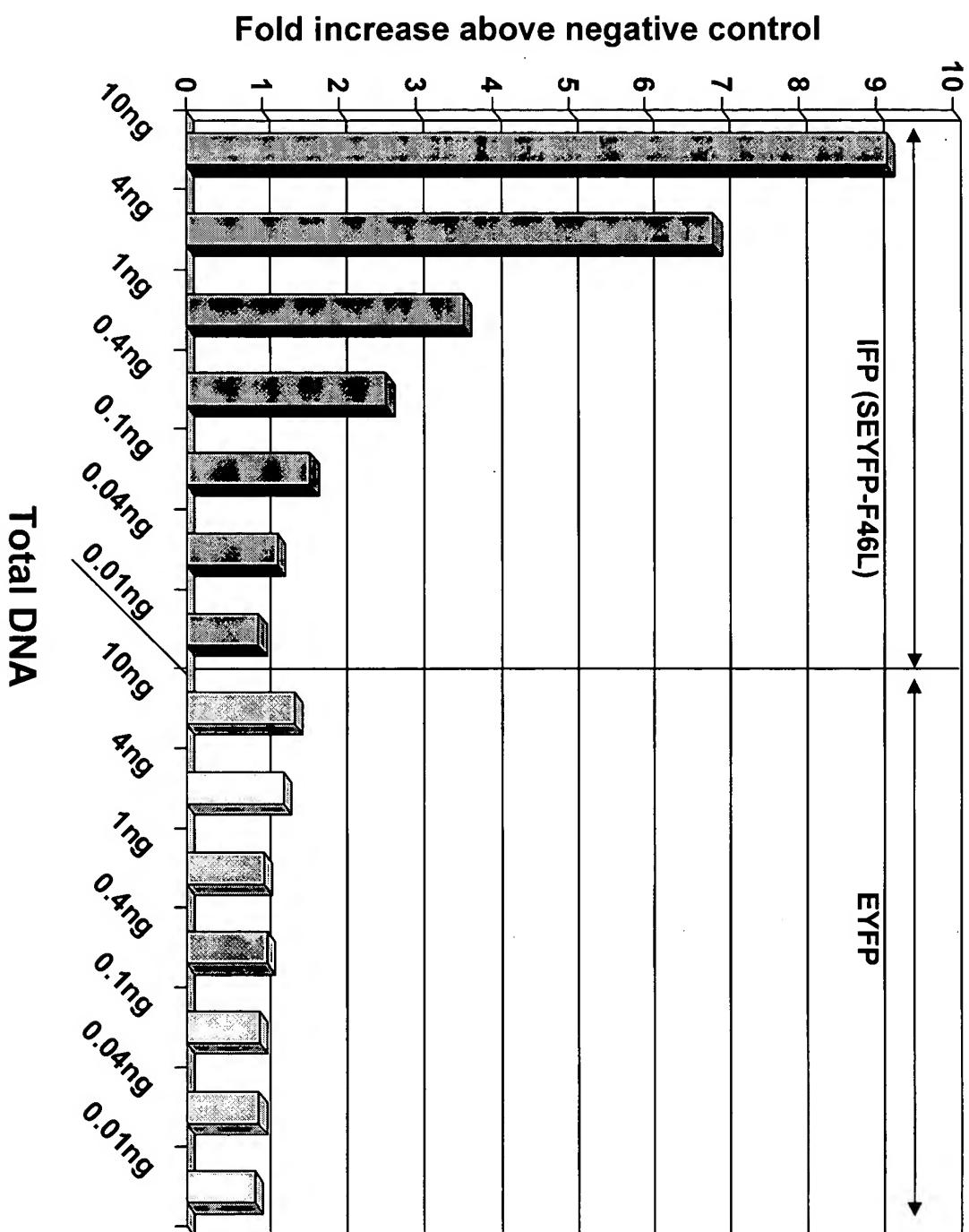


Fig. 8
High-content assays based on IFP PCA:
cytokine-dependent translocation of p65/p50

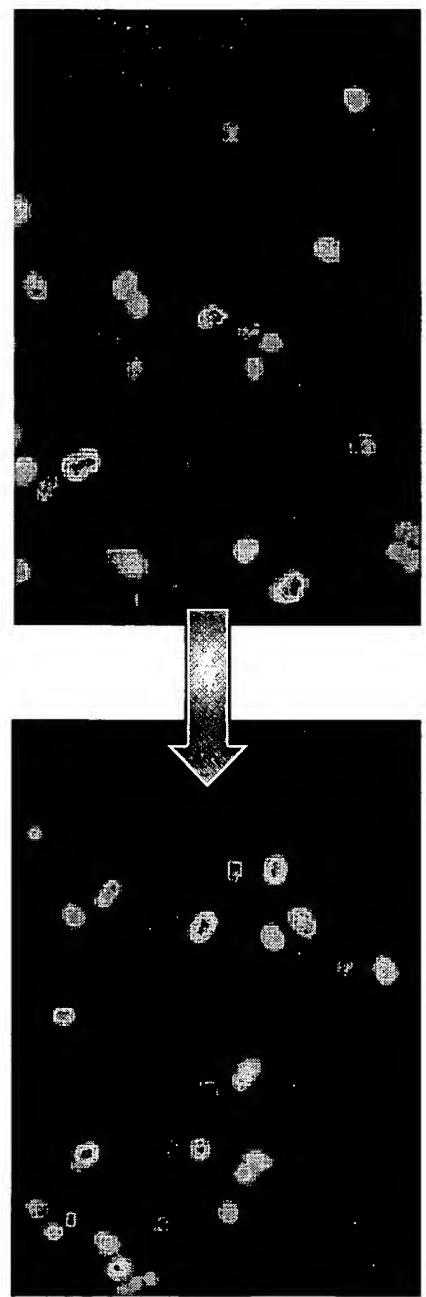
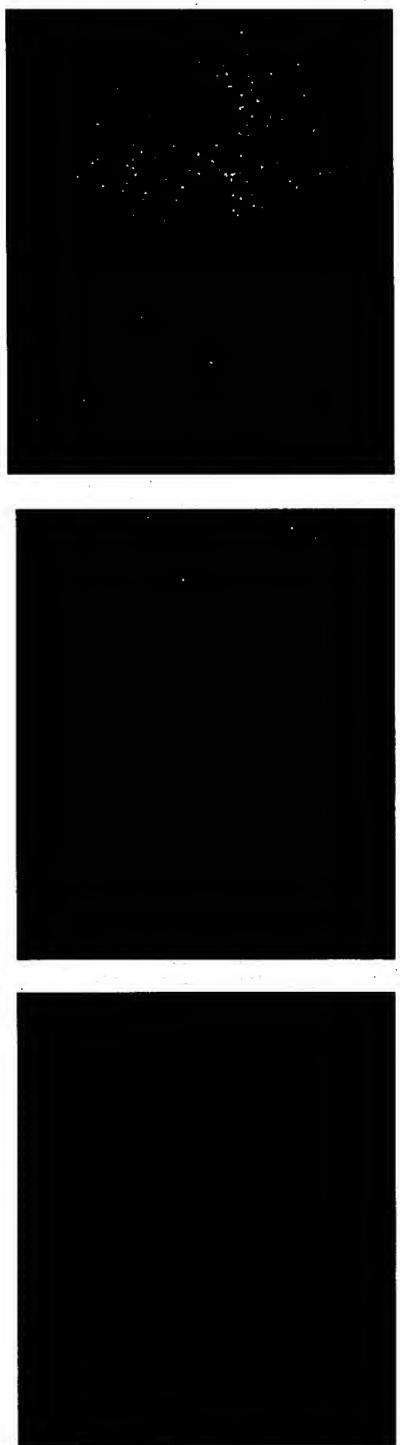
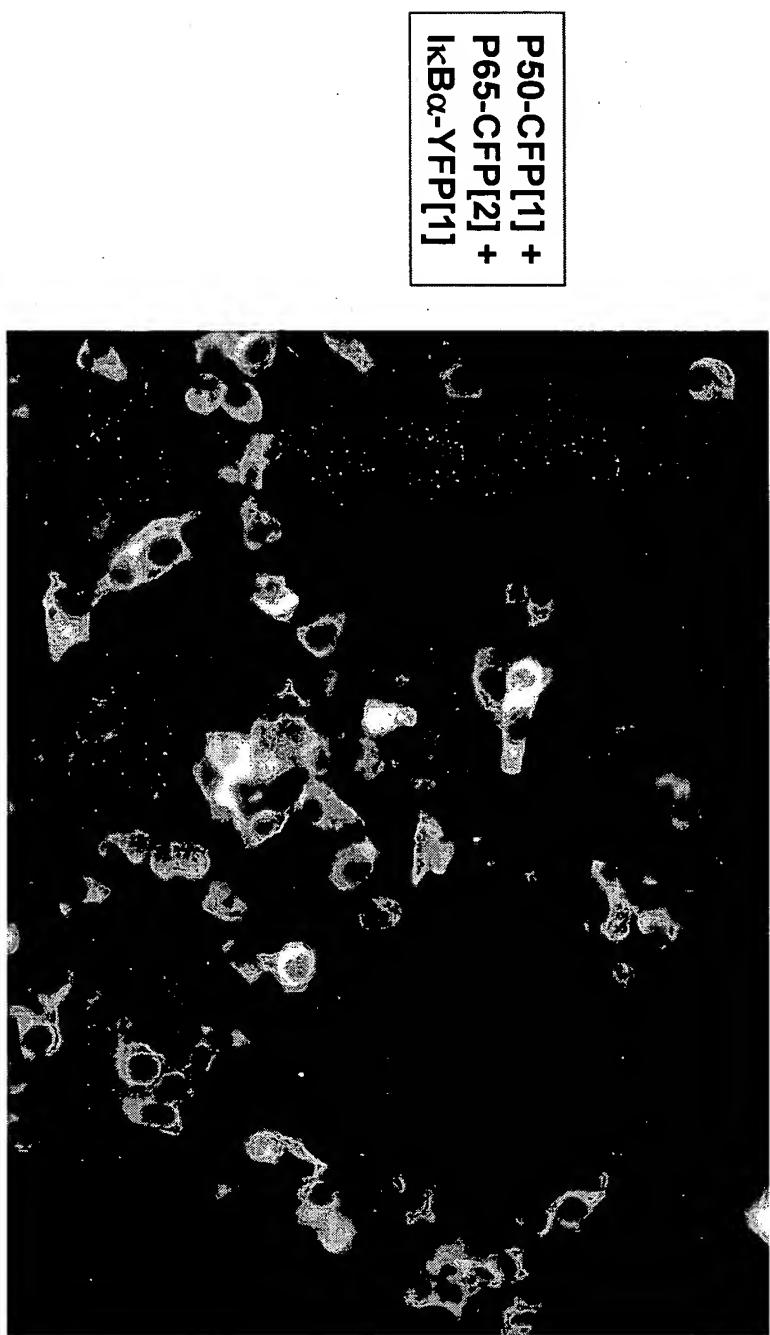


Fig. 9 .
Spectrally shifted (blue) PCAs based on mutant fluorescent protein fragments
(excitation= 436 nm, emission= 480 nm)



14-3-3 σ /14-3-3 σ
p65/p50
Negative Control

Fig. 10.
Multi-color protein-fragment complementation assays (PCA)



CFP channel
Excitation= 426-446 nm
Emission= 460-500 nm

FITC channel
Excitation=460-500 nm
Emission= 505-560 nm